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A new stability indicating RP-HPLC method for estimation of brexpiprazole

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ABSTRACT

A simple stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Brexpiprazole in tablet dosage forms. Reversed-phase chromatography was performed on Shimadzu Model CBM-20A/20 Alite, using a mixture of 0.1% acetic acid and methanol (65:35, v/v) as mobile phase with a flow rate of 0.9 mL/min. Detection was carried at 214nm. Linearity was observed over the concentration range of 0.1–250 µg/mL (R^2 = 0.9999) with regression equation y = 39617.94x + 3300.8. Brexpiprazole was subjected to stress conditions (acidic, alkaline, oxidation and thermal degradation) and validated as per ICH guidelines. The validated method can be applied to perform long-term and accelerated stability studies of Brexpiprazole formulations.

Keywords: Brexpiprazole; Isocratic elution; Reversed-phase HPLC; Stability-indicating; Validation.

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1. INTRODUCTION

Brexpiprazole (BXP), chemically known as 7-{4-[4-(1-benzothiophene-4-yl) piperazin-1-yl] butoxy}-1, 2-dihydroquinoline-2-one is an atypical antipsychotic indicated for the treatment of schizophrenia and adjunctive treatment of MDD [1]. Brexpiprazole (Figure 1) is a partial agonist at 5-HT1A and D2 receptors with similar potency, and an antagonist at 5-HT2A and adrenergic α1B/2C receptors. Compared with aripiprazole, brexpiprazole is more potent at 5-HT1A receptors and displays less intrinsic activity at D2 receptors [2].

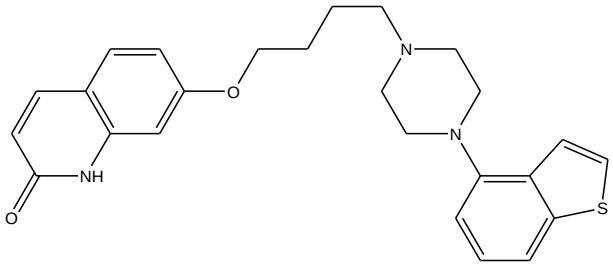


Figure 1: Chemical structure of Brexpiprazole

Literature survey revealed that Brexpiprazole was determined by UV-Visible spectroscopy [3] and HPLC [4, 5]. In the present study, the authors have proposed four simple validated spectrophotometric methods for the determination of Brexpiprazole in pharmaceutical dosage forms. At present, the authors have developed stability indicating RP-HPLC method for the determination of Brexpiprazole in presence of its degradation products.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents:

HPLC grade Methanol (Merck), glacial acetic acid (Merck), sodium acetate trihydrate (Merck), Hydrochloric acid (Rankem), sodium hydroxide (Rankem), Hydrogen peroxide (Rankem) was used. Brexpiprazole, obtained as a gift sample from MSN Life Sciences Pvt. Ltd., (India), was used.

Brexpiprazole is available with brand name Rexulti (0.5 mg and 1mg of the drug per tablet; Otsuka America Pharmaceuticals Inc.) were purchased from the local market.

2.2. Instrumentation and conditions:

Chromatographic separation was achieved by using a Shimadzu Mode.000l CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with Phenomenex C18 (250 mm × 4.6 mm i.d., 5 µm particle size) column kept up at 25 ºC.

2.3. Preparation of stock solution:

Brexpiprazole stock solution (400 µg/mL) was prepared by weighing accurately 10 mg of BXP in a 25 mL volumetric flask with methanol. Working standard solutions were prepared on daily basis from the stock solution with methanol.

2.4. Preparation of 0.1% Acetic acid:

1mL of acetic acid was dissolved in HPLC grade water in a 1000 mL volumetric flask (pH 3.90).

Isocratic elution was performed using 0.1% acetic acid and methanol (65:35, v/v). The overall run time was 10 min. and the flow rate was 0.9 mL/min. 20 µL of sample was infused into the HPLC system.

3. METHOD VALIDATION

The method was validated for system suitability, linearity, and limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, and robustness.

3.1. Linearity:

Linearity test solutions for the assay method were set up from a stock solution at various concentration levels (0.1–250 µg/mL) of the assay analyte concentration and 20 µL of each solution was infused into the HPLC system and the obtained peak area region from the chromatogram was noted. The calibration curve was plotted by taking the concentration on the x-axis and the relating peak area on the y-axis. The data were treated with linear regression analysis method. The limit of quantification and limit of detection depended on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in ICH guidelines Q2 (R1) [6].

3.2. Precision and accuracy:

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of BXP at three concentration levels (10, 20 and 50 µg mL) (n=3) against a qualified reference standard. The %RSD of three acquired assay values at three diverse concentration levels was determined. The inter-day precision study was performed on three different days i.e. day-I, day-II and day-III at three different concentration levels (10, 20 and 50 µg/mL) and each value is the average of three determinations (n=3). The % RSD of the three obtained assay values on three different days was calculated. The accuracy of the assay method was assessed in triplicate at three concentration levels (50, 100 and 150%), and the percentage recoveries were determined. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of BXP in the drug product. The study was carried out in triplicate at 50, 100 and 150 µg/mL. The percentage of recovery in each case was calculated.

3.3. Robustness:

The robustness of the assay method was established by providing small changes in the HPLC conditions which included wavelength (209 and 219 nm), the percentage of methanol in the mobile phase (33 and 37%) and flow rate (0.8 and 1.0 mL/min). Robustness of the method was studied utilizing six replicates at a concentration level of 100 µg/mL of BXP.

3.4. Assay of marketed formulations (Tablets):

Solutions were also prepared by extracting the marketed formulations (Tablets) with the methanol and filtered. The filtrate so obtained was diluted as per the requirement and 20 µL solution of each of the marketed formulations (REXULTI®) was injected into the HPLC system and from the calibration curve, the percentage recovery was calculated.

3.5. Forced degradation studies:

Forced degradation studies were performed to assess the stability indicating properties and specificity of the method. All solutions for stress studies were prepared at an initial concentration of 400µg/mL of BXP and refluxed for 2 hours at 60 ºC and then diluted with methanol.

400µg/mL of BXP solution was exposed to acidic degradation with 0.1 M HCl for 2 hours at 60 ºC the stressed sample was cooled, neutralized and diluted with methanol. Similarly, stress studies were conducted in alkaline conditions with 0.1 M NaOH at 60 ºC for 2 hours and neutralized after cooling with proper dilution with methanol.

Oxidative stress studies were performed using 30 % H2O2 and thermal stress studies were conducted in the thermostat at 60 ºC for 2 hours. 20 µL solution of each of the solutions under forced degradation studies were infused into the HPLC system and the chromatograms were recorded from which the percentage recovery and also the degradants were studied.

4. RESULTS AND DISCUSSION

Initially, the stressed samples were analyzed using a mixture of 0.1% acetic acid and methanol (50:50, v/v). with a flow rate of 0.7 mL/min in which the resolution and peak symmetry was not satisfactory. The flow rate was changed to 0.9 mL/min and the drug sample was injected into the loop where a sharp peak was eluted at 3.545 minutes with tailing. Finally, the mobile phase composition was modified as 65:35, v/v and the drug peak eluted were sharp and symmetrical (UV detection at 214 nm) with retention time less than 5 minutes (2.17 ± 0.03 min). Brexpiprazole shows linearity over a concentration range of 0.1–250 µg/mL (Table 1) with % RSD 0. 24-0.65. The linear regression equation was found to be y = 39617.9x +3300.8 (R2 = 0.9999). The LOQ was found to be 0.0614µg/mL and the LOD was found to be 0.0203µg/mL. The % RSD range was obtained as 0.24-0.45 and 0.14-0.61 for intra-day and inter-day precision studies respectively (Table 2). 99.70-100.14 % of recovery was observed in the accuracy studies with % RSD 0.42-0.62 (<2.0 %) (Table 2).

Table 1: Linearity of Brexpiprazole

Table 1: Linearity of Brexpiprazole		
Conc.	Mean peak area	RSD
0.1	3475±15.6375	0.45
0.5	19654±125.7856	0.64
1	39775±135.235	0.34
10	401587±963.8088	0.24
20	789657±2526.902	0.32
50	2006445±9029.003	0.45
100	3947575±24474.97	0.62
150	5998743±25194.72	0.42
200	7854287±29060.86	0.37
250	9937192±64591.75	0.65

*Mean of three replicates

Table 2: Precision and accuracy studies of Brexpiprazole				
Conc. (µg/mL)	Intra-day precision		Inter-day precision	
	* Mean peak area ± SD (% RSD)		* Mean peak area ± SD (% RSD)	
10	401587±963.8088 (0.24)		401323± 784.36 (0.19)	
20	789657±2526.902 (0.32)		789489± 1078.23 (0.14)	
50	2006445±9029.003 (0.45)		2005789± 12223.17 (0.61)	
Accuracy				
Spiked conc. (µg/mL)	Total conc. (µg/mL)	* Mean peak area ± SD (% RSD)	Drug Found (µg/mL)	% Recovery
25 (50 %)	50	2006369±9185.007 (0.46)	49.85	99.70
75 (100 %)	100	3947489±24378.47 (0.62)	99.84	99.84
125(150 %)	150	5998813±25384.52 (0.42)	150.21	100.14

*Mean of three replicates

The robustness of an analytical procedure refers to its capacity to stay unaffected by small or deliberate variations in method parameters and gives an indication of its reliability for routine analysis. The robustness of the method was evaluated by testing a similar sample under various analytical conditions deliberately changing from the first condition. The outcomes obtained (Table 3) from the assay of the test solutions were not influenced by varying the conditions and were as per the results for unique conditions. The % RSD value of assay for a similar sample under original conditions and robustness conditions was under 2.0% (0.29-0.64) showing that the developed method was robust.

The representative chromatogram, 3D Curve and Peak Purity Curve of Brexpiprazole were shown in Figure 2A, 3A, 4A. The proposed method was applied to the marketed formulations (REXULTI®) and the percentage recovery was 98.41-100.14 (Table 4) without the interference from the excipients used in these tablets (Figure 2B-2C, 3B-3C& 4B-4C).

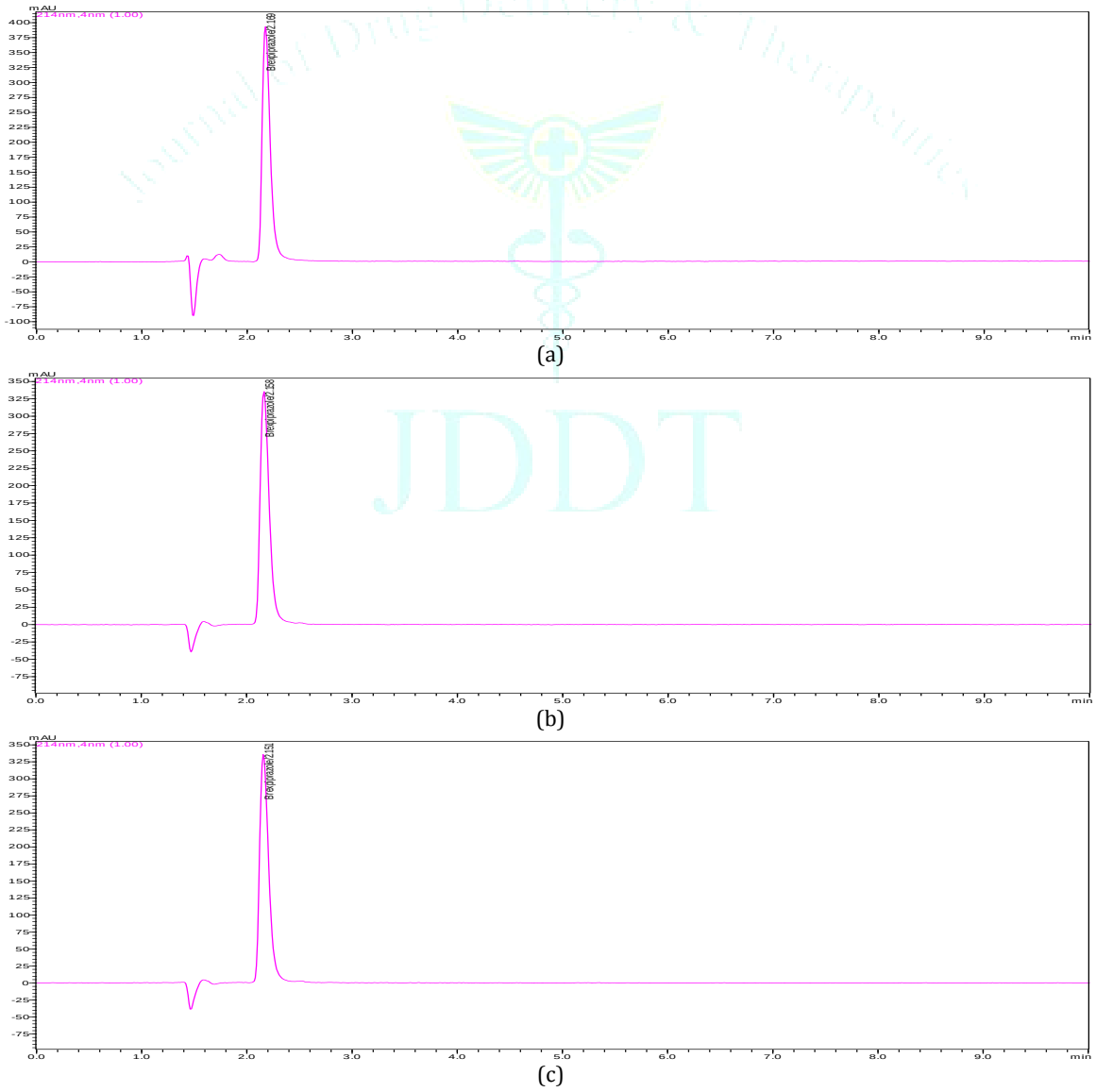
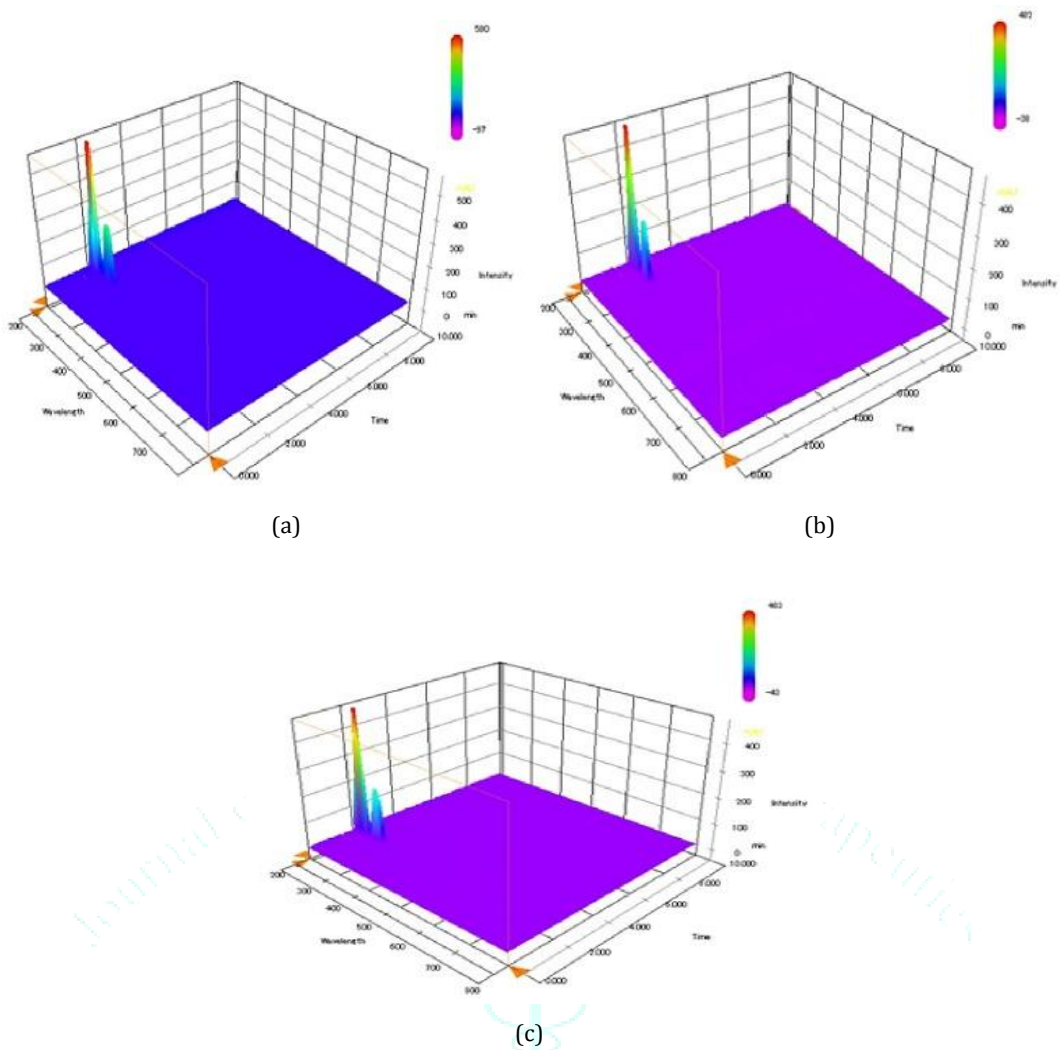


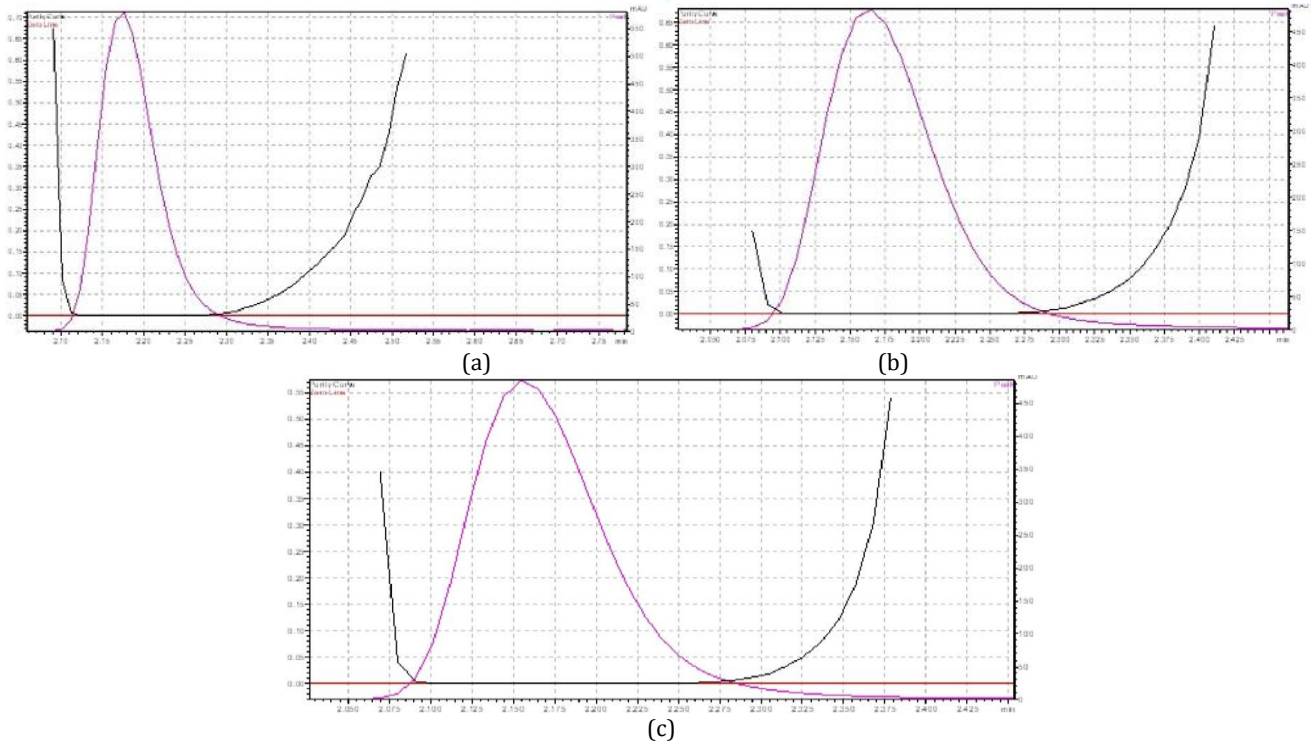
Figure 2: Typical chromatograms of Brexpiprazole (a) Standard Drug (10 µg ml-1) (b) Formulation 1: REXULTI® (Label claim: 0.5 mg) and (c) Formulation 2: REXULTI® (Label claim: 1.0 mg).



(a) (b)

(c)

Figure 3: Typical 3D curves of Brexpiprazole (a) Standard Drug (10 µg ml-1) (b) Formulation 1: REXULTI® (Label claim: 0.5 mg) and (c) Formulation 2: REXULTI® (Label claim: 1.0 mg).



(a) (b)

(c)

Figure 4: Peak Purity curves of Brexpiprazole (a) Standard Drug (10 µg ml-1) (b) Formulation 1: REXULTI® (Label claim: 0.5 mg) and (c) Formulation 2: REXULTI® (Label claim: 1.0 mg).

Table 3: Robustness study of Brexpiprazole

Parameter	Condition	*Mean peak area	*Mean peak area ± SD (% RSD)	*Assay (%)
Flow rate (± 0.9 mL/min)	0.8	3941418	3961253.33 ± 23718.48 (0.59)	99.84
	0.9	3954816		
	1.0	3987526		
Detection wavelength (± 5 nm)	209	3942422	3936972.33 ± 23221.16 (0.59)	98.91
	214	3956984		
	219	3911511		
Mobile phase composition (0.1% acetic acid: methanol) (± 2 %, v/v)	63:37	3951875	3959336.00 ± 25332.39 (0.64)	100.01
	65:35	3938572		
	67:33	3987561		
pH (± 0.1 unit)	3.8	3965871	3974062.33 ± 11779.59 (0.29)	99.71
	3.9	3987562		
	4.0	3968754		

*Mean of three replicates

Table 4: Analysis of Brexpiprazole commercial formulation (Tablets)

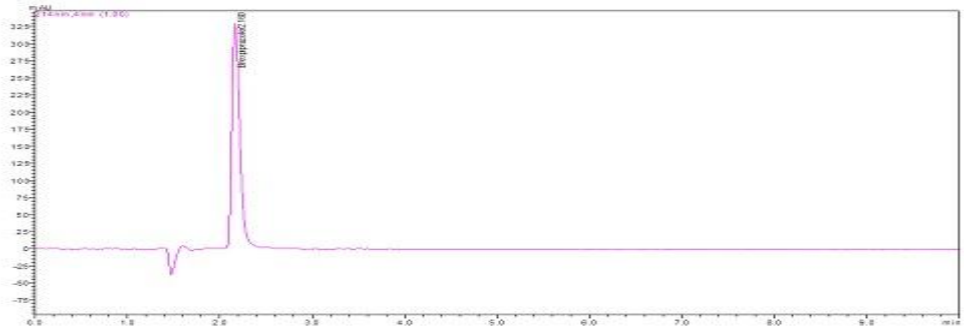
Sample No.	Formulation	Labeled claim (mg)	*Amount found (mg)	*Recovery (%)
1	Brand I	0.5	0.496	99.20
1	Brand II	1.0	0.994	99.40

*Mean of three replicates

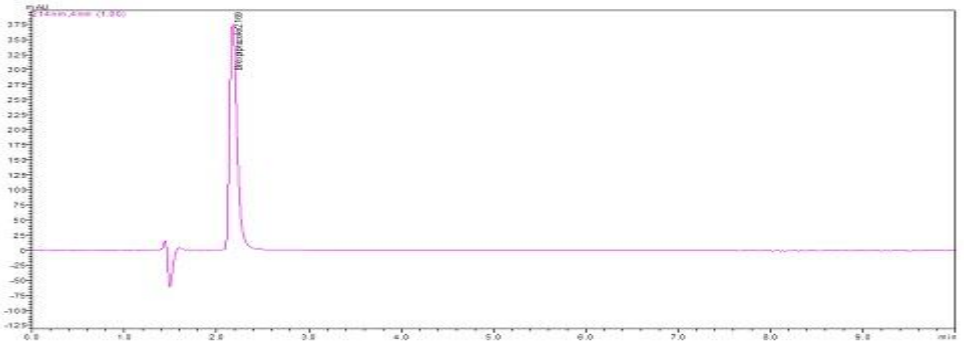
The stability indicating the capability of the method was established from the separation of BXP peak from the degraded samples. The degradation of BXP was found to be very similar for both the tablets and standard. Typical Chromatogram 3D curves and peak purity curves chromatograms obtained following the assay of stressed samples is shown in Figure 5A-5E, 6A-6E, and 7A-7E respectively. A slight decomposition was seen on exposure of BXP drug solution to acidic (0.14 %), alkaline (0.18 %), oxidative (8.64 %) and thermal (0.86 %) conditions. The degradants were observed at 1.585, 1.757 and 1.986 min in oxidation degradations without interfering with the Brexpiprazole peak indicating that the method is specific.

The percentage of drug degradation was less than 10% in all stressed conditions indicating that Brexpiprazole is very much resistant (Table 5).

The present stability-indicating method for the determination of BXP in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. Also, Brexpiprazole is more resistant towards all degradations and the overall data demonstrated that the excipients and the degradation products did not interfere with the BXP peak. The system suitability parameters for the BXP peak shows that the theoretical plates were more than 2000 and the tailing factor was less than 2 (or <1.5-2.0) (Table 5).



(a)



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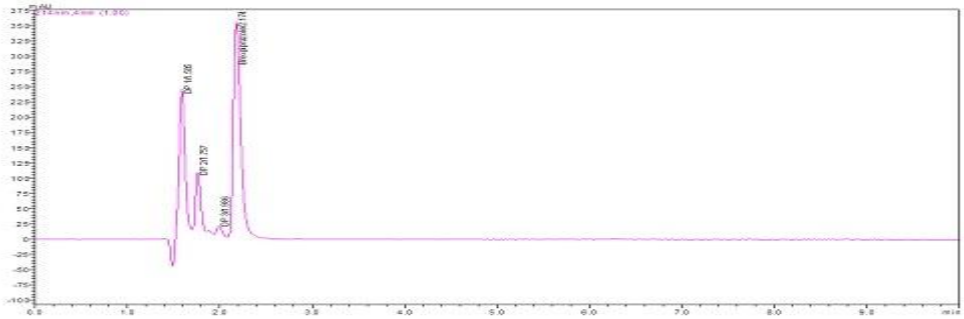

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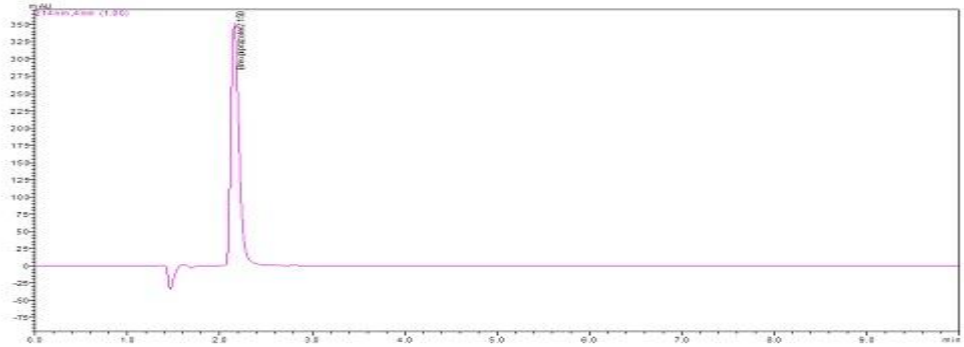
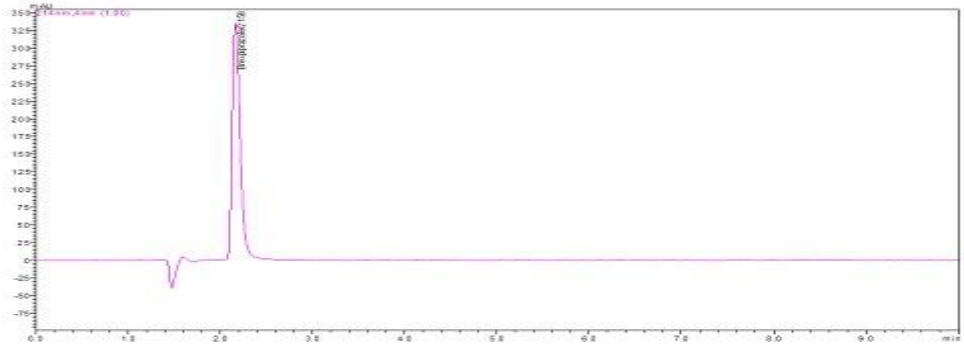


(d)



(e)

Figure 5: Typical Chromatograms of Brexpiprazole (10µg/ml) on (a) Acidic degradation, (b) Alkaline degradation, (c) Oxidative degradation and (d) Thermal degradation and (e) Photolytic degradation.

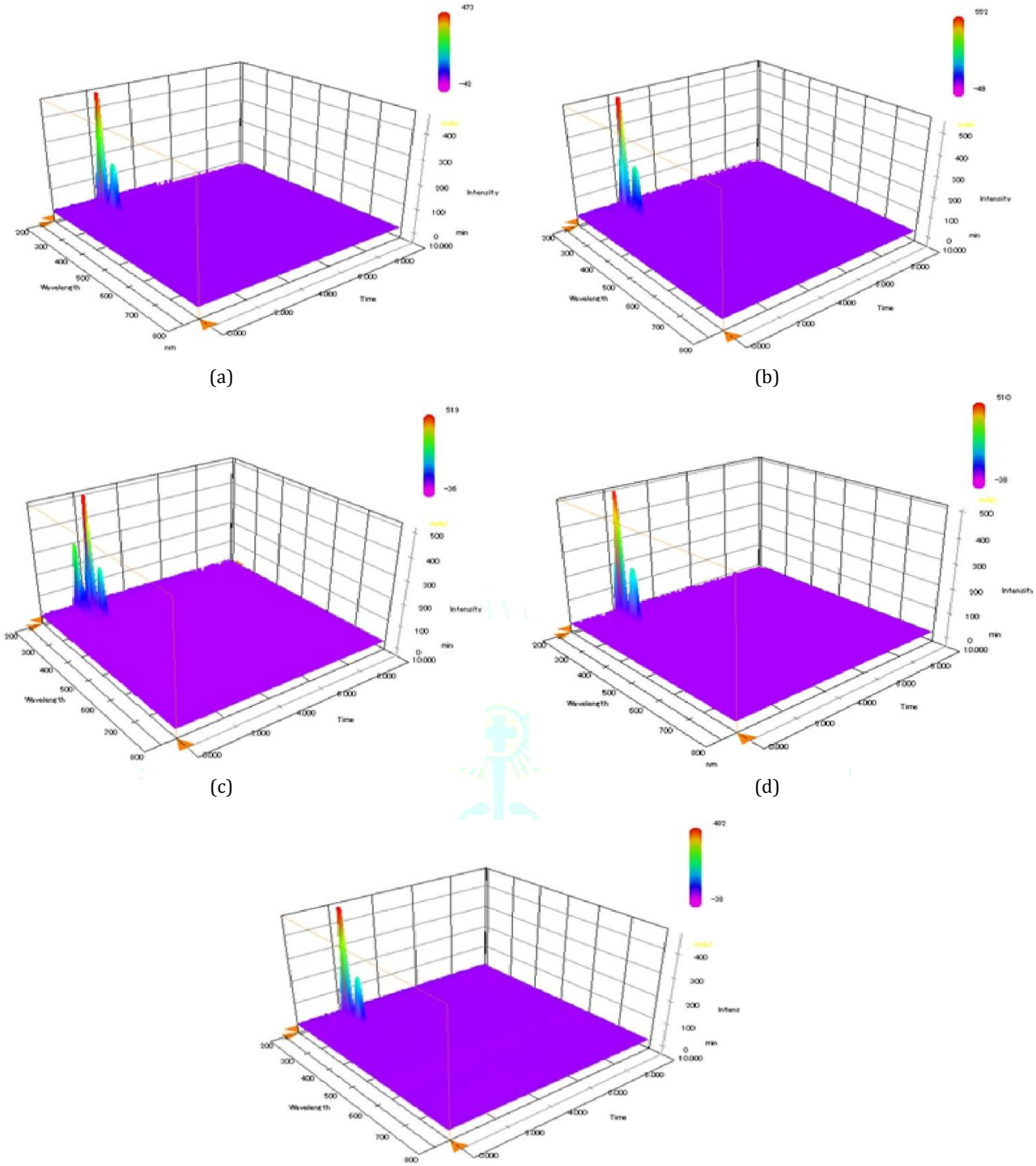


Figure 6: Typical 3D Curves of Brexpiprazole (10µg/ml) on (a) Acidic degradation, (b) Alkaline degradation, (c) Oxidative degradation and (d) Thermal degradation and (e) Photolytic degradation.

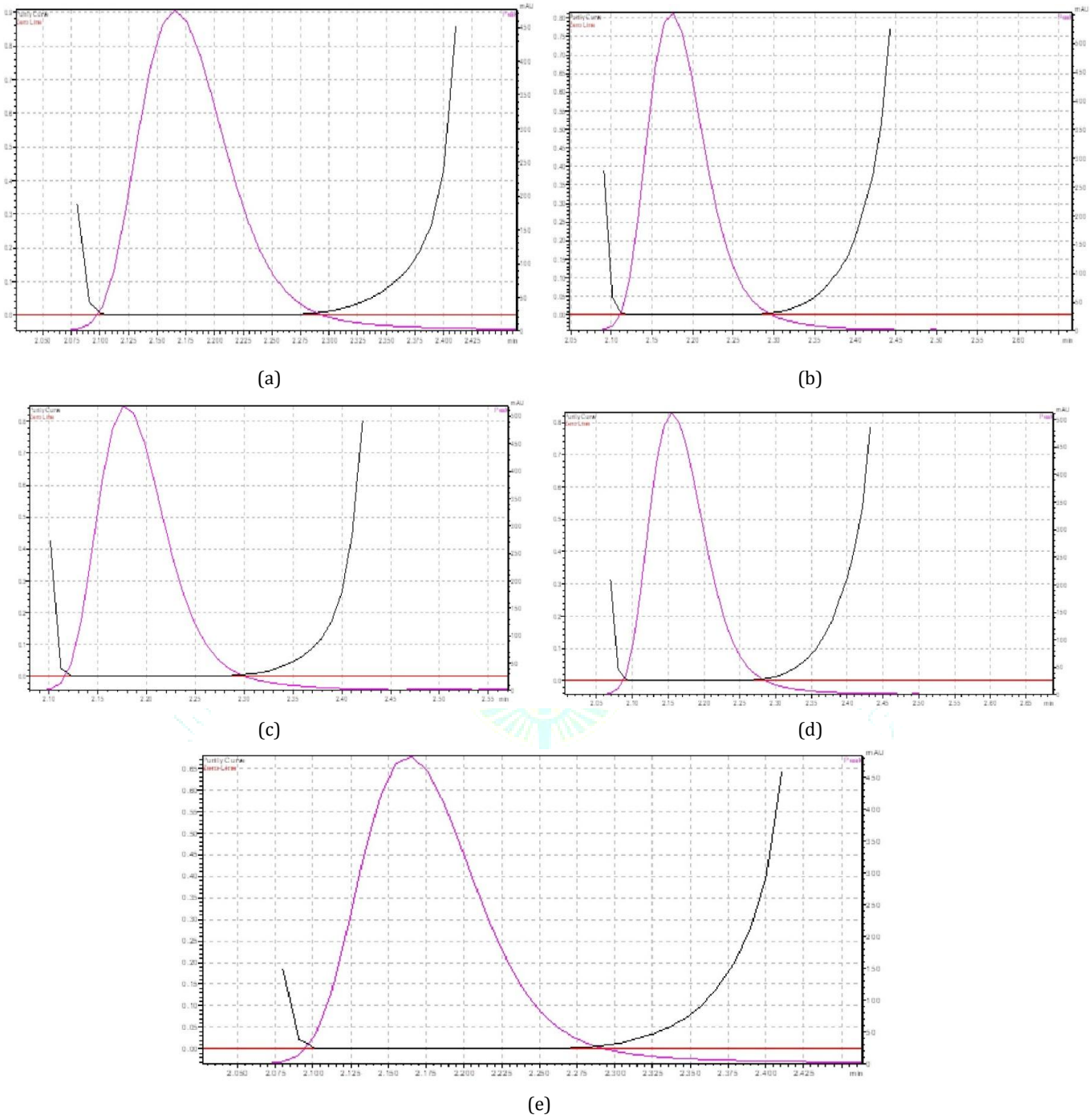


Figure 7: Peak Purity Curves of Brexpiprazole (10µg/ml) on (a) Acidic degradation, (b) Alkaline degradation, (c) Oxidative degradation and (d) Thermal degradation and (e) Photolytic degradation.

Table 5: Forced degradation studies of Brexpiprazole

Stress Conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug (Untreated)	3947575	100	-	7548.15	1.254
Acidic degradation 1 ml 0.1N HCl, 80°C, 30 mins	3942048	99.86	0.14	7365.19	1.287
Alkaline degradation 0.1 ml 0.1N NaOH, 80°C, 30 mins	3940469	99.82	0.18	7247.36	1.282
Oxidative degradation 1 ml 3% H_2O_2, 80°C, 30 mins	3606504	91.36	8.64	7258.36	1.267
Thermal degradation 105°C, 30 mins	3913626	99.14	0.86	7247.96	1.258

*Mean of three replicates

5. CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of Brexpiprazole in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of Brexpiprazole formulations.

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CONFLICT OF INTEREST

There is no conflict of interest between authors.

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